Enantioselective Binding of α-Pinene and of Some Cyclohexanetriol Derivatives by Cyclodextrin Hosts: A Molecular Modeling Study*

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ABSTRACT

We have used molecular modeling to investigate the enantioselective separation of the monoterpene α-pinene on permethylated β-cyclodextrin and on α-cyclodextrin and the enantioselective separation of three cyclohexanetriol derivatives on permethylated β-cyclodextrin. Using the Consistent Valence Force Field (CVFF) from Insight/Discover, we have carried out systematic rigid-body docking grid searches on each of the optical antipodes of the organic guest molecules interacting with the cyclodextrins, followed by minimizations of the low-energy docked structures. A statistical mechanical analysis of the minimized energies yields data that agree in four out of five cases with the experimental elution order of enantiomers. The computed energies of the rigid-body docking before minimizations do not agree with the experimental results, suggesting that a conformational induced fit of the cyclodextrins upon binding of the organic guests may be involved in the mechanism of the chiral recognition.

Introduction

The terpene α-pinene, one of the most widespread of the bicyclic monoterpenes, is a chiral compound obtained from oil of turpentine. In North American oils the dextrorotatory enantiomer predominates, but in European oils the levorotatory enantiomer dominates.[1] α-Pinene is used industrially as a solvent (turpentine) and in the manufacture of such products as camphor and synthetic pine oil.[1]

α-Pinene enantiomers also exhibit interesting biological and stereochemical properties. The major monoterpene constituent in the defensive secretions of termites (*Nasutitermes princeps*) has been

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identified as dextrorotatory α-pinene with 99.5% optical purity.[2] The red turpentine beetle exhibits a stereospecific antennal response to α-pinene enantiomers,[3] and the chirality of α-pinene is important in the beetles' host finding behavior.[4] Analysis of the *Bupleurum fruticescens* essential oil, which exhibits antiinflammatory properties, revealed α-pinene as one of two major components.[5] α-Pinene is also a predominant component in a drug used in Europe for treatment of liver and kidney diseases.[6] An area of increasing importance involving α-pinene is directed chiral synthesis.[7] Recently, α-pinene has been used in the asymmetric synthesis of L-699, 392, a leukotriene antagonist.[8] Syntheses involving α-pinene as a chiral precursor establish a need for samples of the compound in a highly pure form.[9]

An important method for separating enantiomers involves cyclodextrins.[10] Cyclodextrins are macrocyclic molecules formed from α-(1 → 4)-linked D-glucopyranose units. The oligomers, composed of six or more glucose units, adopt a toroid shape. The resulting cavity gives the cyclodextrins well characterized complexing properties.[10] Cyclodextrins have found uses in the pharmaceutical industry as encapsulation agents and as tools for research in catalysis and in the separation sciences.[11] The inherent chirality of the cyclodextrin molecules allows them to form diastereomeric complexes with enantiomeric compounds. Cyclodextrins and their derivatives, therefore, are useful tools in inducing asymmetric reactions,[12] and have been used extensively as stationary phases in chromatographic enantioselective separations of a wide variety of chiral compounds. The often dramatic difference in biological effects of the enantiomers of a chiral drug demonstrates the need for enantioselective stationary phases, such as cyclodextrin, to obtain optically pure compounds.[13]

Specifically, permethylated β-cyclodextrin[14-19] and α-cyclodextrin[20] have been used in gas chromatography to separate enantiomers of α-pinene. Also, permethylated β-cyclodextrin has similarly been used to separate enantiomers of several cyclohexanetriol derivatives.[21] In this study, we use molecular modeling to explore the chromatographic separation of α-pinene (**I** in Figure 1) racemates by permethylated β-cyclodextrin and by α-cyclodextrin (see Figure 2) and to explore the separation of enantiomers of three 2,3-isopropylidene-1,2,3-cyclohexanetriol derivatives (**II**, **III**, and **IV** in Figure 1) by permethylated β-cyclodextrin. The cyclohexanetriols are important intermediates in natural product total synthesis.[22]

α-Pinene is of particular interest in enantiomeric separations due to its hydrocarbon structure and lack of polar functional groups. It serves as a model for studying enantioselective interactions of cyclodextrins with unfunctionalized molecules. Enantiomeric separation of the nonpolar solute limonene has been previously modeled successfully.[23]

In contrast to α-pinene, the cyclohexanetriols serve as models in enantiomeric separations due to their polar structure and presence of functional groups. By modeling the enantiomers of α-pinene and of cyclohexanetriols, we will hope to elucidate the mechanism of separations of small, rigid, cyclic organic molecules with nonpolar or polar structures.

In this molecular modeling study we make the assumption that the guests bind primarily within the cavity of the cyclodextrins. This point, however, is subject to some debate because some chiral separations occur even when no cavity is available. For example, separations of polar guest molecules have been successfully performed on open-chained polysaccharides.[24] However, Kobor et al.[25] have pointed out that, while chiral recognition by interaction with the outer surface of a cyclodextrin molecule may be important in separating highly polar solutes, formation of inclusion complexes is necessary for chiral recognition of nonpolar molecules. Keeping this in mind, we recognize that some interactions leading to chiral discrimination

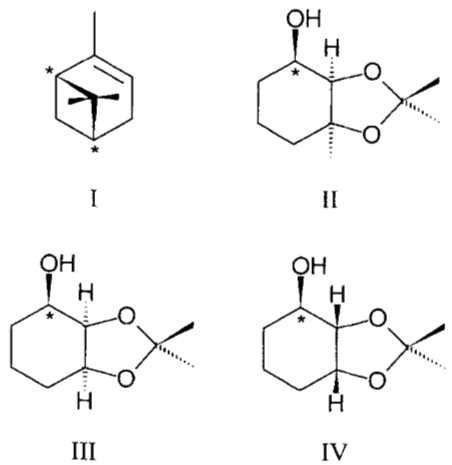


FIGURE 1. Guest molecules. Only the *R* enantiomers are shown. **I**, **II**, **III**, and **IV** are a-pinene and three derivatives of 2,3-isopropylidene-1,2,3-cyclohexanetriol derivatives. The asterisk (*) marks the chiral carbon.

of the cyclohexanetriols may occur outside the cavity. However, the well known propensity of cyclodextrins to form inclusion complexes[26-29] and previous success at modeling enantioselective binding of polar solutes in cyclodextrin cavities[26,30-33] suggest a high probability of complexation of the organic guests (both polar and nonpolar) in the cyclodextrin cavity. Therefore, the systems that we model in this study will be a one-to-one complexation of the guest molecules within the cyclodextrin cavity.

Our attempt is to correctly predict elution order for enantiomeric separations by demonstrating lower average energies for the appropriate diastereomeric complexes formed between the enantiomers and the cyclodextrins.

Methodology

Molecular modeling was performed on a Silicon Graphics Indigo 2 workstation using the CVFF force field of Biosym Insight/Discover.[34] Interaction energies obtained in the docking grid searches were calculated using the Insight II docking module. Energy minimizations were performed using the conjugate gradients method to a derivative of 0.01 kcal mol^{-1}. Boltzmann averages of energies were evaluate at 300 K. The differences between the Boltzmann averages of the total energy for the diastereomeric complexes were used to compute the $\Delta\Delta G$ of binding. All calculations were performed in vacuo, that is, solvent effects were neglected. The justifications for this are (1) we compare the relative stabilities of systems with the guests already complexed into the cyclodextrin cavity, (2) the effect of the solvent is expected to be the same for enantiomers, and (3) the enantioselective separations being modeled were performed in the gas phase.

MODELING HOST AND GUEST MOLECULES

To model the permethylated β-cyclodextrin, we used the published coordinates of the X-ray crystal structure of a one-to-one *p*-iodophenol:permethylated β-cyclodextrin complex.[35] Starting with the cyclodextrin structure only (i.e., leaving out the *p*-iodophenol), we minimized the total conformational energy to obtain the permethylated β-cyclodextrin structure used in subsequent calculations. The minimization produced little change in the structure.

To model α-cyclodextrin in our docking grid searches, we used the following procedure. With Insight II, we generated and then cyclized a six-member D-glucose oligomer with α(1 → 4) glycosidic links, and then used Insight/Discover to minimize the total conformational energy to yield an initial low-energy α-cyclodextrin. We then used this minimum-energy conformation as the starting structure for a 60-ps NVT molecular dynamics simulation to explore several conformational states.

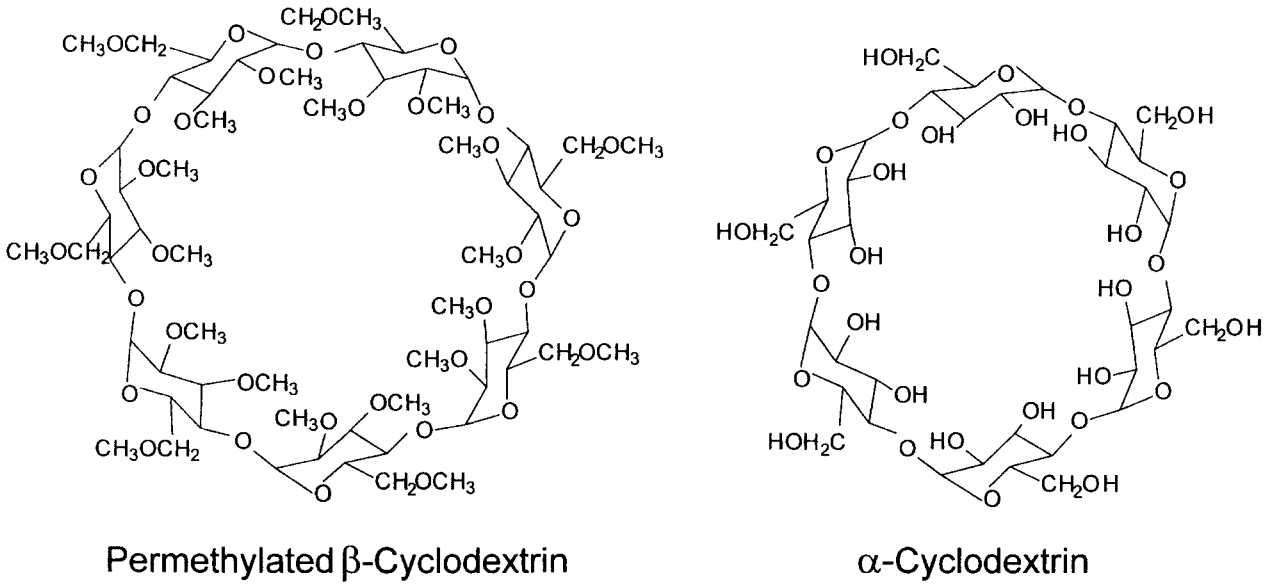


FIGURE 2. Host molecules.

To obtain the final structure for the grid searches, we determined the average conformation over the last 50 ps of the simulation and minimized that structure.

To model the α-pinene and cyclohexanetriol enantiomers, we generated their structures with the Insight II builder module, then minimized the total conformational energy. The results yielded rigid bicyclic structures, making it necessary to use only one conformation of each enantiomer in the docking calculations.

RIGID-BODY DOCKING GRID SEARCHES

The optical antipodes of α-pinene were moved systematically in transrotational space through an extended cylindrical volume within the cavity of the permethylated β-cyclodextrin. The dimensions and placement of the cylinder were defined to allow a thorough search of the cavity space while preventing an excess of high-energy overlapping configurations. The conformations of the guest and host molecules were kept rigid throughout the grid search. The docking configuration of the guest molecule was defined by three translational and two rotational parameters relative to the host molecule.[36] Dappen et al.[37] reported that, in docking grid searches, a 1.0-Å translational resolution and a 24° rotational resolution were too coarse to find all the important low-energy configurations. Therefore, we used a 0.5-Å translational resolution and a 15° rotational resolution. Our initial grid search explored a cylindrical space with elliptical width of 5.0 Å, height of 4.5 Å, and a depth of 4.0 Å. At each translational–rotational grid locus, a guest–host interaction energy was determined.

The interaction energies obtained from the docking simulations were then used to refine the cylindrical docking space in the permethylated β-cyclodextrin, and a second tighter grid search was performed. The dimensions of the cylindrical space searched in the second grid search were 2.0 Å in both width and height and 1.0 Å in depth. The translational and rotational resolutions were 0.25 Å and 12°, respectively. A Boltzmann average of the resulting interaction energies for each grid search was then calculated. The lowest-energy configurations in both runs for both enantiomers placed the α-pinene molecules at the center of the host β-cyclodextrin cavity.

Enantiomers of **II**, **III**, and **IV** were systematically docked in transrotational space into the cavity of permethylated β-cyclodextrin using the same protocol as followed above docking α-pinene into permethylated β-cyclodextrin. The extended cylindrical space searched had an elliptical width of 5.0 Å, height of 4.5 Å, and a depth of 4.0 Å. The translational and rotational grid resolutions were 0.5 Å and 15°. A Boltzmann average of the resulting interaction energies for each grid search was calculated.

The smaller size of the α-cyclodextrin cavity greatly limits the translational freedom of the α-pinene guest molecules. Thus, instead of searching an extended elliptical volume within the α-cyclodextrin cavity, the guest molecules were moved systematically through the center of the cavity space from 10 Å above the α-cyclodextrin center of mass to 10 Å below the center of mass. The translational and rotational resolutions of this docking grid search were 0.25 Å and 8°. Interaction energies were measured at each transrotational position of the guest molecule, and a Boltzmann average was taken of the energies for each α-pinene enantiomer.

MINIMIZATIONS OF HOST–GUEST COMPLEXES

We used low-energy structures from the docking studies as starting conformations for further energy minimization to obtain final conformations and energies of host–guest complexes. The procedure involved selecting the 500 lowest-energy configurations for each α-pinene and cyclohexanetriol enantiomer docked into permethylated β-cyclodextrin and minimizing to a derivative of 0.01 kcal mol^{-1} using the conjugate gradients method. These minimizations yielded extensive degeneracy in the sets of minimized inclusion complexes. We then sorted the minimized complexes and analyzed for uniqueness by heavy-atom root-mean-square (rms) positional superimposition, using a threshold value of 0.05 Å as the rms deviation to determine uniqueness. Thus, we obtained lists of unique structures with accompanying energies for each enantiomer of α-pinene and of the three cyclohexanetriol derivatives in permethylated β-cyclodextrin. We used a similar method to obtain lists of unique structures for each enantiomer of α-pinene in α-cyclodextrin.

Results and Discussion

Using molecular modeling to compute very small energy differences is a difficult task, but in this case, we are modeling the differences in con-

formational and interactional energies of nearly identical systems. We can therefore make the assumption that computational errors in the complexes of one enantiomeric complex will effectively cancel out errors in the other, thus producing very small but potentially meaningful energy differences.[36,38]

RIGID-BODY DOCKING GRID SEARCHES

Boltzmann average energies for the rigid-body docking grid searches of α-pinene and cyclohexanetriols in permethylated β-cylodextrin and α-pinene in α-cyclodextrin are presented in Table I. In four out of five cases, the elution order of the enantiomers was incorrectly predicted. This suggests that the approximations involved in a rigid-body grid search preclude accurate modeling of the fine interactions necessary for chiral discrimination. The error from rigid-body docking has been previously shown to be too large for modeling enantiomeric separations.[37]

MINIMIZATIONS

Our preliminary work indicated that a rigid-body docking grid search is not sufficiently accurate to model enantiomeric separations, but it can serve as an effective method for selecting starting points for further refining through energy minimizations. The results of our minimizations of low-energy docked structures are presented in Table II. The energy ranges shown in the table include complexes with both enantiomers. The difference in the Boltzmann averages for the two α-pinene enantiomers bound to permethylated β-

TABLE I.

Results from the Systematic Rigid-Body Docking Grid Search for Enatiomers of α-Pinene (I) and Three Cyclohexanetriol Derivatives (II, III, and IV) Interacting with Permethylated β-Cyclodextrin (PM β-CD) or α-Cyclodextrin (α-CD) Host Cavities.

Guest Host	I PM β-CD	II PM β-CD	III PM β-CD	IV PM β-CD	I α-CD
Boltzmann average (*R*) enantiomer (kcal mol^{-1})	−20.6	−21.7	−21.0	−19.6	−11.8(0)
Boltzmann average (*S*) enantiomer (kcal mol^{-1})	−21.9	−19.8	−22.2	−20.0	−11.7(7)
$\Delta\Delta G$ (kcal mol^{-1})	1.3	1.9	1.2	0.4	0.0(3)
Experimental tightest bound	*R*	*S*	*S*	*R*	*S*
Predicted tightest bound	*S*	*R*	*S*	*S*	*R*

TABLE II.

Results from the Minimizations of Low-Energy Rigid-Body Docked Structures of Enatiomers of α-Pinene (I) and Three Cyclohexanetriol Derivatives (II, III, and IV) Interacting with Permethylated β-Cyclodextrin (PM β-CD) or α-Cyclodextrin (α-CD) Host Cavities.

Guest Host	I PM β-CD	II PM β-CD	III PM β-CD	IV PM β-CD	I α-CD
Unique inclusion complexes with (*R*) enantiomer	18	49	45	63	23
Unique inclusion complexes with (*S*) enantiomer	20	60	46	54	20
Energy range (kcal mol^{-1})	297.6–302.3	172.7–181.8	197.0–207.6	188.5–202.7	216.1–223.7
Boltzmann average (*R*) enantiomer (kcal mol^{-1})	298.1	173.8	197.9(6)	188.8	216.6
Boltzmann average (*S*) enantiomer (kcal mol^{-1})	298.2	173.1	197.9(5)	190.6	216.4
$\Delta\Delta G$ (kcal mol^{-1})	0.1	0.7	0.0(1)	1.8	0.2
Experimental tightest bound	*R*	*S*	*S*	*R*	*S*
Predicted tightest bound	*R*	*S*	*S* ~ *R*	*R*	*S*

cyclodextrin yields a $\Delta\Delta G$ of 0.1 kcal mol^{-1}, which correctly predicts the *R* enantiometer to be bound tighter. Tighter binding of the *R* enantiomer of **II** was correctly predicted with a $\Delta\Delta G$ value of 0.7 kcal mol^{-1}. The elution order was also correctly predicted for **III**, with the *S* enantiomer binding tighter by 1.8 kcal mol^{-1}. The elution order prediction for **IV** was inconclusive. The correct **IV** enantiomer was predicted to be bound tighter, but only by 0.01 kcal mol^{-1}.

The changes in cyclodextrin structure with minimization were small, but they were enough to allow the host molecule to adjust and better accommodate the guest molecule. This suggests that, to some degree, the interaction between the guest enantiomers and permethylated β-cyclodextrin may be considered an induce fit, where the conformation of the host changes to produce a better fit. The importance of induced-fit behavior in cyclodextrin complexation has been previously demonstrated experimentally and theoretically.[39,40]

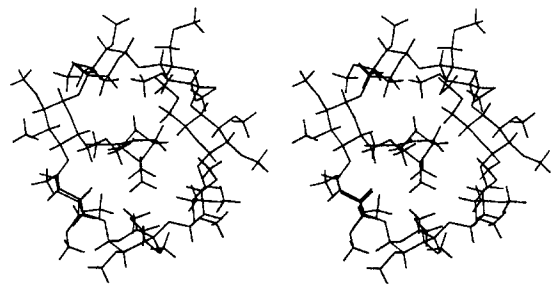
Docking of α-pinene into α-cyclodextrin yields a $\Delta\Delta G$ of 0.2 kcal mol^{-1}, as shown in Table II. Experimentally, *S*-α-pinene is observed to elute last when separated on α-cyclodextrin and thus should be bound more tightly. The calculated Boltzmann averages indicate that the *S*-α-pinene:α-cyclodextrin dastereomeric complex is indeed more stable.

As with the permethylated β-cyclodextrin, the rigid-body docking grid searches predicts incorrect order of elution, while the minimizations, which allow full molecular relaxation, predict the correct elution order. With α-cyclodextrin, the change in conformation upon minimizing the energy is small but significant, again suggesting the importance of an induced-fit mechanism of binding.

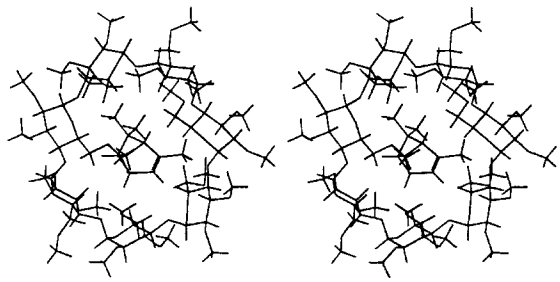
We also note that the lowest-energy minimum of the *R*-α-pinene:α-cyclodextrin complex is lower than that of the lowest-energy minimum of the *S*-α-pinene complex. Thus, comparing just the global minimum-energy structure would incorrectly predict the elution order. However, when the entire ensemble of structures is used to determine Boltzmann averages, the correct elution order is predicted. This indicates the complexity of the interactions involved in chiral recognition and demonstrates the importance of considering not just the global minimum, but a large portion of the energy surface.

The structures of the global minimum-energy complexes for enantiomers of α-pinene in permethylated β-cyclodextrin and in α-cyclodextrin are shown in Figures 3 and 4. The global minimum-energy complexes for enantiomers of **IV** in permethylated β-cyclodextrin are shown in Figure 5. In both cyclodextrins the guest molecules are complexed into the center of the cavity. However, α-pinene appears to be more deeply sequestered into permethylated β-cyclodextrin than into α-cyclodextrin. Negative docking energies obtained from the rigid-body docking calculations and from the minimizations indicate that complexation of guests into the cyclodextrin cavities is highly favored.

To explore what interactions are present in the complexes that lead to the observed and calculated chiral discrimination, we examined low-energy minimized complexes for both cyclodextrins. The absence of polar and hydrogen-bondable groups on α-pinene implies that its stereodifferential binding arises from differences in steric interactions with the host cyclodextrin. However, these steric differences are not present in the lowest-energy structures, because with permethylated β-cyclodextrin, the lowest-energy α-pinene complexes are essentially identical in energy and with α-cyclodextrin, the lowest energy complexes predict the wrong order of elution, while in both cases

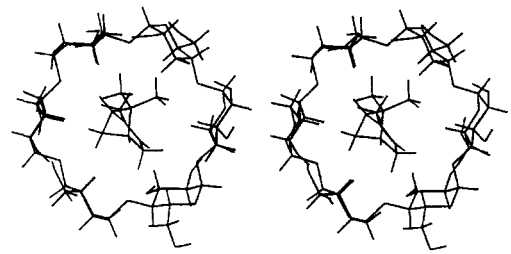


R-**I** in permethylated β-cyclodextrin

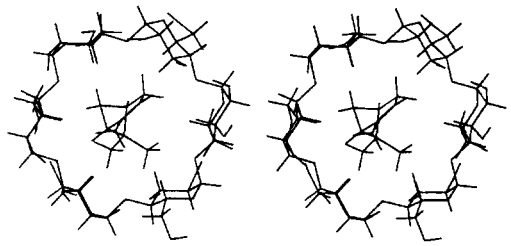


S-**I** in permethylated β-cyclodextrin

FIGURE 3. Stereo plots of lowest-energy minimized inclusion complexes of α-pinene with permethylated β-cyclodextrin.

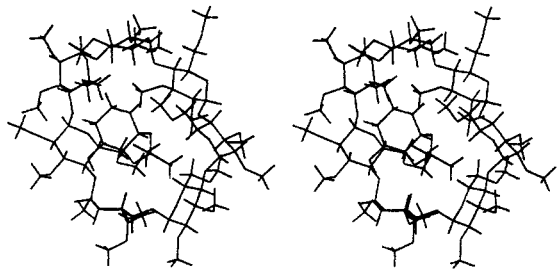


R-I in α-cyclodextrin

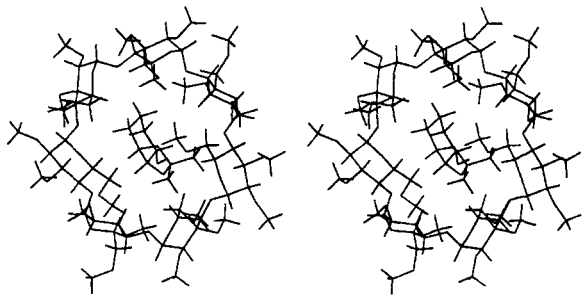


S-I in α-cyclodextrin

FIGURE 4. Stereo plots of lowest-energy minimized inclusion complexes of α-pinene with α-cyclodextrin.



R-IV in permethylated β-cyclodextrin



S-IV in permethylated β-cyclodextrin

FIGURE 5. Stereo plots of lowest-energy minimized inclusion complexes of **IV** with permethylated β-cyclodextrin.

the Boltzmann averages predict the correct order of elution. This suggests that the energy surface is too complex to use a three-point or other simple interaction model to explain the mechanism of enantioselectivity.

To understand the mechanism of enantioselectivity, therefore, we examined not only the lowest-energy complexes, but other low-energy minimized complexes as well, keeping in mind the view that chirality is a property of an entire molecule, not just its chiral center(s).[41]

In all the low-energy guest–host complexes, the enantiomers of α-pinene bind at the center of, and deep into, the permethylated β-cyclodextrin cavity. Within the cavity, the methyl groups of α-pinene consistently dock into pockets formed by the methoxy substituents of three adjacent ring glucose moieties. These observations are in general agreement with the modified Armstrong cyclodextrin chiral recognition model of Lipkowitz et al.,[26] except that unfunctionalized α-pinene lacks the ability to form a strong interaction with the lip of the cyclodextrin cavity. Beyond the general observations that we have already noted, we cannot describe the host–guest interactions using a simple model because of the complexity and subtlety of the numerous contacts resulting in the chiral recognition.

In the low-energy complexes of the cyclohexanetriol derivatives with permethylated β-cyclodextrin, the guest enantiomers are bound deep into the cyclodextrin cavity. The single hydroxyl group on the cyclohexanetriols consistently forms a hydrogen bond with the cyclodextrin host.

Analysis of the low-energy α-cyclodextrin complexes shows that two orientations of α-pinene are prevalent in the minimized complexes. These orientations are distinguished by which portion of the α-pinene molecule is pointed inward toward the center of the cavity—either the vinyl methyl group and alkene moiety or the two methyl groups on carbon 6. The lower-energy configurations of both enantiomers are dominated by the first of these orientations, while the higher-energy configurations are dominated by the latter. The *S* enantiomer of α-pinene appears to be able to adopt the latter orientation at a lower energy than does the *R* enantiomer. This results in a clustering of minimum-energy structures that have energies near that of the global minimum: *S*-α-pinene binding to α-cyclodextrin yields five complexes with energies within 1.0 kcal mol^{-1} of the global minimum,

while *R*-α-pinene binding yields only three complexes within 1.0 kcal mol^{-1} of the global minimum. Thus, while the *R*-α-pinene global-minimum complex is lower in energy than the *S*-α-pinene global-minimum complex, the Boltzmann average energy of *S*-α-pinene is lower than that of *R*-α-pinene.

It is interesting to note that similar studies have been performed using rigorous molecular dynamics simulations.[30,33] In those studies, the less favorably placed substrate could be observed to move out and return into the cavity in the most convenient way. In this study, the rigor of the grid search allows exploration of many orientations of the guests within the host cavity, and the most favorable orientations are selected by choosing low-energy configurations for minimization.

Conclusions

The calculated results of this molecular modeling study are in agreement with experimental observation in predicting the correct elution order in four out of five systems. In the other system, the calculations predicted no resolution. Thus far, this modeling scheme has yielded correct predictions on 80% of the systems studied, which have involved four pairs of guest enantiomers and two host molecules.

An interesting result of this study is the dramatic difference in the results of the rigid-body grid searches versus the minimizations. In four of the five systems studied, the elution order was incorrectly predicted by the rigid-body grid search, but correctly predicted by the minimizations, which allowed full relaxation of the complexes. We take this as additional evidence that rigid-body grid searches introduce an unacceptable amount of error into chiral recognition calculations. Furthermore, these results offer additional support to previous work implicating the importance of induced fit in cyclodextrin complexation[39,40] and also suggest that an induced-fit mechanism may be important in chiral recognition by cyclodextrins. The potential energy surface of cyclodextrin appears to be too complex for describing chiral recognition by a simple interaction model. This study also provides further evidence of the importance of considering the entire energy surface and not just the putative global minima in interpreting molecular modeling data in chiral recognition studies.

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